SLOVENSKI STANDARD
SIST EN 16847:2016

01-december-2016

Gnojila - Določevanje kompleksirajočih agensov v gnojilih - Prepoznavanje heptaglukonske kisline s kromatografijo

Fertilizers - Determination of complexing agents in fertilizers - Identification of heptagluconic acid by chromatography

Düngemittel - Bestimmung von Komplexbildnern in Düngemitteln - Bestimmung von Heptaglukonsäure mit Chromatographie

Engrais - Détermination des agents complexants dans les engrais - Identification de l'acide heptagluconique par chromatographie

Ta slovenski standard je istoveten z: EN 16847:2016

ICS:

65.080 Gnojila Fertilizers

SIST EN 16847:2016 en,fr,de

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 16847

January 2016

ICS 65.080

English Version

Fertilizers - Determination of complexing agents in fertilizers - Identification of heptagluconic acid by chromatography

Engrais - Détermination des agents complexants dans les engrais - Identification de l'acide heptagluconique par chromatographie

Düngemittel - Bestimmung von Komplexbildnern in Düngemitteln - Identifikation von Heptaglukonsäure mit Chromatographie

This European Standard was approved by CEN on 17 November 2015.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

European foreword

This document (EN 16847:2016) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by July 2016, and conflicting national standards shall be withdrawn at the latest by July 2016.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

1 Scope

This European Standard specifies a chromatographic method which allows the identification of heptagluconic acid (HGA) in fertilizers containing heptagluconic acid metal complexes.

This method is applicable to EC fertilizers containing complexed micro-nutrients, which are covered by Regulation (EC) No 2003/2003 [1].

NOTE For the complete names of the chelating agents mentioned in this document, see Annex E.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12944-1:1999, *Fertilizers and liming materials and soil improvers — Vocabulary — Part 1: General terms*

EN 12944-2:1999, *Fertilizers and liming materials and soil improvers — Vocabulary — Part 2: Terms relating to fertilizers*

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 12944-1:1999 and EN 12944-2:1999 apply.

4 Principle

The method is based on demetalation with phosphoric acid of the micronutrient HGA complex present in an aqueous solution of the sample.

The complexing agent is then identified and determined by high-performance liquid chromatography.

The separation is carried out on an NH_2 phase bonded to silica column and an aqueous solution of phosphoric acid and acetonitrile as eluent.

The detection is based on UV photometry at 210 nm.

5 Interferences

a) High concentrations of phosphate in the sample solution can create a large peak preventing the identification/determination of HGA.

b) High concentrations of chloride, sulfate and nitrate do not interfere in the identification/determination of the complexing agent.

c) The presence of the chelates of EDDHSA, [*o,o*]EDDHA, [*o,o*]EDDHMA, EDTA, DTPA, CDTA, HEEDTA, IDHA as well as the corresponding chelating agents do not interfere since they are separated from HGA.

   These substances can be detected in the chromatogram by the appearance of a peak at larger retention times. Therefore, the presence of these kinds of substances shall be taken into account when successive injections are scheduled.

d) The presence of gluconic acid does interfere in the determination of the complexing agent.

e) The presence of aspartic acid, humic substances and lignosulfonic acid may interfere with the identification/determination of HGA.

6 Apparatus

Usual laboratory equipment, glassware, and the following:

6.1 Magnetic stirrer.

6.2 Chromatograph,

equipped with:

a) an isocratic pump delivering the eluent at a flow rate of 1 ml/min;

b) an injection valve with a 20 µl injection loop or equivalent;

c) a NH_2 column; internal diameter: 4,6 mm; column length: 250 mm; dp = 5 µm [1];

d) a NH_2 guard-column (recommended);

e) a UV-Vis detector with a 210 nm-filter;

f) an integrator.

6.3 Chromatographic conditions,

according to Table 1.

Table 1 — Chromatographic conditions

Flow rate	Eluent A (7.4) %	Eluent B (7.5) %
1 ml/min	75	25

6.4 Balance,

Balance, with an accuracy of ± 0,1 mg.

6.5 Membrane filters.

Micro membrane filters resistant to aqueous solutions, with porosity of respectively 0,45 µm and 0,2 µm.

7 Reagents

Use only reagents of recognized analytical grade.

[1]) Phenosphere NH_2 80A 5 µm 250×4,6 mm or equivalent. This is an example of suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products can be used if they can be shown to lead to the same results.

7.1 Water,

conforming to EN ISO 3696, degassed by boiling before use.

7.2 Sample preparation solvent.

Add to 800 ml of water, 2 ml of *ortho*-phosphoric acid 85 % and 25 ml of methanol in a 1 l volumetric flask. Dilute to the mark with water and homogenize.

7.3 HGA stock solution,

$c(HGA_{acid})$ = 19 893 mg/l.

This solution shall be freshly prepared daily, because of the formation of the corresponding lactone if it let standing for a long period of time.

Weigh to an accuracy of 0,1 mg about 2 500 mg of the heptagluconic acid, sodium salt dihydrate (CAS # 10094-62-9), c > 99 %, add 50 ml of water in a 100 ml volumetric flask. After dissolution, dilute to the mark with water and homogenize.

7.4 Eluent A: *ortho*-phosphoric acid,

$c(H_3PO_4)$ = 30 mmol/l and methanol.

Add to 800 ml of water, 2 ml of *ortho*-phosphoric acid 85 % (mass concentration) and 25 ml of methanol (HPLC grade) in a 1 l volumetric flask. Dilute to the mark with water and homogenize. Before use, filter the solution through a 0,45 µm membrane filter (6.5).

7.5 Eluent B: acetonitrile (HPLC-grade).

8 Procedure

8.1 Preparation of the HGA-metal complex sample solution

The mass of the test portion to be used to prepare the sample solution is dependent on the declared metal content of the product.

NOTE Sample preparation may be conducted according to EN 1482-2, see [6].

Weigh into a 150 ml beaker, approximately the amount of sample specified in Table 2, to an accuracy of 0,1 mg:

Table 2 — Amount of sample

Declared metal content % (mass fraction)	Mass of test portion mg
10 to 15	300
5 to 10	500
< 5	1 000

Add 50 ml of sample preparation solvent (7.2) and dissolve it with a magnetic stirrer (6.1) during 5 min. Make up to volume in a 100 ml volumetric flask with sample preparation solvent (7.2). Let the solution stand overnight in darkness to allow the metal phosphate to form.

8.2 Preparation of the calibration solutions

Pipette a volume (*V* ml) (see Table 3) of the HGA stock solution (7.3) in six 100 ml volumetric flasks respectively. Make up to volume with the sample preparation solution (7.2) and homogenize. Let the solution stand overnight.

Table 3 — Composition of the calibration solutions

Solution	*V* ml	Concentration of HGA (acid) mg HGA/l
1	1	199
2	2	398
3	6	1 194
4	8	1 591
5	10	1 989
6	16	3 183

NOTE The molecular mass of heptagluconic acid, sodium salt dihydrate corresponds to 284 g/mol, whereas the acid form has a molecular mass of 226 g/mol.

8.3 Chromatographic analysis

Immediately before injection, all calibration and sample solutions shall be filtered through a 0,2 µm membrane filter (6.5).

After stabilization of the chromatographic conditions (6.3), inject the calibration solutions (8.2) into the chromatographic system (6.2).

The major peak obtained corresponds to heptagluconic acid.

NOTE 1 Since the calibration solutions are not freshly prepared (see 8.2), two defined peaks may appear in the chromatograms, one tentatively assigned to the lactone and the other corresponding to the heptagluconic acid.

Adjust the attenuation on the integrator, in order to obtain a suitable range for the HGA peak from the standard solution. A typical chromatogram is given in Figure A.1. Measure the retention time.

Draw the calibration curve with the value of the chromatographic peak of the calibration solutions versus the HGA (acid) concentration (mg/l) in the standards.

Inject the sample solution (8.1). Identify the complexing agent by the retention time of the obtained peaks, and if diode array detector is used, confirm it with its UV-visible spectrum (see Annex B).

Measure the area of the peak for the sample solution corresponding to the complexing agent and determine the concentration in (mg/l) using the calibration graph. See Annex A for integration considerations.

NOTE 2 Heptagluconic acid can co-exist in two different isomers: alpha and beta. Both isomers can be found in commercial products. The retention times of both isomers differ in less than 0,3 min and they can be distinguished by two separated peaks depending on the type of column used.

NOTE 3 The first part of some chromatograms could present a set of peaks that can disturb dramatically the measurement of the value of the HGA peak. This effect can be observed in e.g. the copper complex or in mixtures of complexes.